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Exploitation of Biomass to the Integrated Production of Bioethanol and Poly(hydroxyalkanoate)s

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Abstract

Fossil fuels are a major source of energy worldwide and serve as raw materials for the production of plastics. However, they have disadvantages such as uneven distribution, price instability, limited availability, and environmental impact. Consequently, there is a need to find alternatives to fossil fuels for both energy and polymer production in the short term. This review focuses on the synthesis of poly(hydroxyalkanoate)s (PHAs), a type of biopolymer, using different bioethanol stream wastes. PHAs exhibit properties comparable to petroleum-based plastics, making them promising replacements. There has been a significant increase in research studies exploring alternatives to fossil fuels and synthetic polymers, as evidenced by the growing number of publications. While biopolymers currently account for only 1% of global polymer production derived from petroleum, the PHA industry is experiencing rapid growth. The market value of PHAs was estimated at $ 168.9 million in 2020, and it is projected to reach $ 440 million by 2031, indicating a compound annual growth rate of 9.2%. The production of this biopolymer is already contributing to an expanding industrial value chain, which is expected to further growth with increased availability of commercial PHAs.

Keywords Biofuels · Biopolymers · Biorefinery · By-products · Sustainable production

Introduction

Over the last 50 years, there has been a substantial rise in energy consumption, primarily driven by population growth and the industrialization of nations. This surge in energy demand has been predominantly met by crude oil, which has served as the primary resource. Fossil fuels account for approximately 80% of the global final energy consumption, encompassing various energy applications such as electricity generation, transportation, and heating [1]. Despite the widespread use of fossil fuels, their consumption poses significant environmental challenges, including global warming and air pollution. These problems have a significant negative impact on human health and the overall well-being of individuals. Additionally, the uneven distribution of fossil fuel resources creates economic disparities among nations, limiting the growth potential of developing countries [2, 3]. Moreover, nonrenewable resources are limited in supply and cannot be used sustainably, compromising this and future generations. The volatility and fluctuation of markets and prices associated with fossil fuel utilization also have adverse economic consequences.

Besides energy, from fossil reserves, it were obtained monomers that are submitted to polymerization processes to synthetize polymers, which are employed to fabricate massive products like plastics. Consequently, the production of plastics heavily relies on fossil fuel-derived feedstocks, constituting around 99% of the total. This represents a significant portion, approximately 8–9% of the global oil and gas consumption [4]. Furthermore, there is an anticipated rise in global demand for conventional plastics, along with an increase in production capacity. Plastics desirable properties as durability and stability became them in indispensable materials into today's lifestyle and are use in different product areas including clothing, medical, and electronic

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industries. From the global plastic production, only 9% is recycled or reused, the remaining residues are incinerated, buried, or left in the environment [5]. Being resistant to degradation processes, plastic are accumulate in marine and terrestrial ecosystems causing environmental problems [6, 7]. Moreover, the fragmentation of larger plastic particles due to various factors such as physical, chemical, biological, and environmental leads to the formation of microplastics. The presence of these particles poses a significant environmental threat due to the potential toxicity of the polymer constituents [8]. For these reasons, new alternatives that reduce economic and social environmental negative impacts, associated with non-renewable energy systems and fossil fuel-based plastics, are looking for governments. In recent decades, various policies have been implemented and supported to promote the exploration of renewable energy sources and the development of sustainable alternatives to traditional plastics through different mechanisms of assistance [2].

In this context, numerous research studies have been conducted with the aim of finding alternatives and replacing both fossil fuels and synthetic polymers. This increased interest is reflected in the number of publications from 2018 to 2022, as it was reported by Scopus base data (Fig. 1). Over the past 5 years, there has been a remarkable increase 1.8 times in the number of publications, indicating the growing drive in the exploration of sustainable solutions.

Analyzing the number of documents per year, from 2018, an exponential growth is observed. According to this, it can be mentioned the two most cited articles within this period (2018–2022). In 2018, Talekar et al. [9] proposed an integrated biorefinery concept that utilized waste pomegranate peels through hydrothermal processing and yeast fermentation to produce bioethanol from glucose. Another significant study conducted in the same year by Kuglarz et al. [10] focused on the development of an integrated production process for cellulosic bioethanol and succinic acid from rapeseed straw, following a dilute-acid pretreatment. Moving on to 2019, Dávila et al. [11] explored the hydrothermal processing of vine shoots for bioethanol production and the extraction of lignin for value-added applications. Patsalou et al. [12] made advancements in 2019 by developing a low environmental impact biorefinery that utilized citrus peel waste for the production of ethanol and methane. The subsequent years also witnessed groundbreaking research, including the use of ultrasound as an auxiliary energy to enhance pretreatments and biorefinery processes for the production of biofuels and chemicals from lignocellulosic biomass [13], as well as the investigation of novel biorefinery approaches for the conversion of chicken manure mixed with rapeseed straw through anaerobic co-digestion and digestate recycling [14]. The contributions continued in 2021 with Saadatinavaz et al. [15] utilizing orange waste for biobutanol and biohydrogen production using an acetone-butanol-ethanol fermentation process within a biorefinery framework. Battista et al. [16] investigated a cascade biorefinery in the same year, aiming to produce biodiesel from coffee oils, fermentable sugars derived from cellulose and hemicellulose, and biomethane from the residual solid fraction after sugar extraction. In 2022, Soltaninejad et al. [17] explored the valorization of potato peel wastes for bioethanol and biogas production through a biorefining process, while Patel et al. [18] employed microalgae as a source of proteins and advanced biofuels, cultivated on volatile fatty acids instead of pure glucose. These studies collectively represent the continuous advancements and innovative approaches within the field of biorefinery, showcasing its potential for sustainable and environmentally friendly solutions in the biofuel industry.

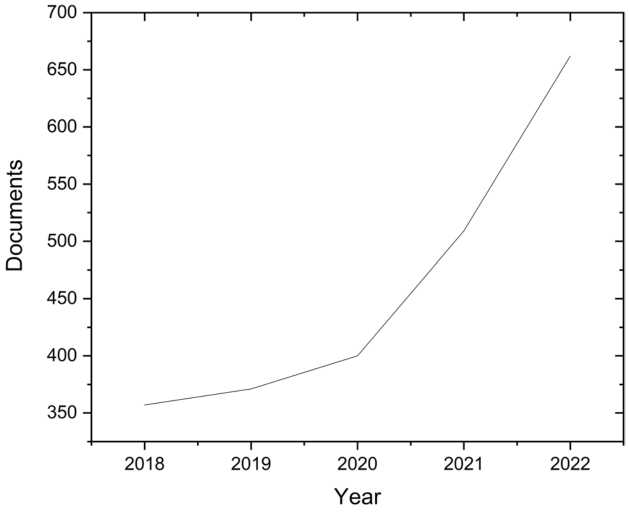


Fig. 1 Documents from the search result by year from 2018 to 2022

As it was evidenced in all of the cited articles, biofuels can be produced by biotechnological processes from agricultural feedstocks instead of geological processes involved in fossil fuels [19]. The carbon dioxide (CO_2) generated by biofuels is treated as atmospheric CO_2 in the plants photosynthetic cycle. Since the agricultural feedstocks employed for biofuel production use CO_2 in their photosynthesis process, contribute to reduce the concentration of this greenhouse gas (GHG) in the atmosphere [20]. Biofuels are nontoxic, sulfur-free, and have biodegradable nature [21–23]. Furthermore, biofuels are derived from readily available biomass sources, which are more evenly distributed geographically compared to fossil fuels. This characteristic enables a more self-reliant and secure energy supply [24].

As it was aforementioned, plastics derived from synthetic polymers are responsible of severe impacts on the environment. Muneer et al. [25] reported that if plastic pollution continues, there would be more plastic particles in the ocean than fish by 2050. Therefore, it is necessary to search new materials that can replace synthetic polymers.

Friendly Environmental Alternative Fuels

Biofuels are categorized into different generations based on the feedstocks utilized, production processes, and employed technologies (Fig. 2a). First-generation biofuels are derived from edible food crops like sugarcane, corn, soybean, sunflower, canola, and wheat, which need fertile agricultural land for cultivation. These biofuels have demonstrated a net positive impact in terms of reducing greenhouse gas emissions and achieving energy balance. For instance, the emissions reduction achieved by sugarcane ethanol compared to gasoline ranges from 59 to 82% [26]. Nevertheless, the production of these biofuels has produced a concern with food security, particularly in developing countries [27]. In order to address this limitation, the biofuel industry has made progress towards second-generation biofuels, which are derived from non-edible organic components of plants such as straw, wood, and agricultural residues [28]. A pretreatment is required for second-generation biomass increasing biofuel cost and represents the most energy consumption during the process of biomass conversion [29]. Several challenges hinder the widespread adoption of second-generation biofuels. These barriers include technological limitations in the production process, high production costs, and the need for accurate monitoring of large-scale projects. Additionally, the type of soil and the geographical location chosen for cultivating biofuel feedstocks can significantly impact the viability and success of second-generation biofuel production [30]. Sustainability of first- and second-generation biofuels is a concern because of the competition with food production and the use of water and other resources for the biomass growth. Another issue is the biodiversity loss and soil erosion. The third-generation biofuels are produced from algae biomass such as microalgae and macroalgae. Biofuels produced from algae feedstocks can reduce considerable GHG emissions [31]. However, third-generation biofuels are not economically competitive with fossil fuels because of their high cost. Fourth-generation feedstocks are genetically modified microalgae and the obtaining of biofuels from these raw materials is in early stages. The fourth-generation biofuels have the low environmental impact [26]. While first-generation ethanol production is already well-established on an industrial scale, the production of second-generation ethanol is still in the development stage. Researchers and industrials are actively exploring various alternatives and approaches for second-generation ethanol production, taking into consideration the specific characteristics and resources available in different regions [32].

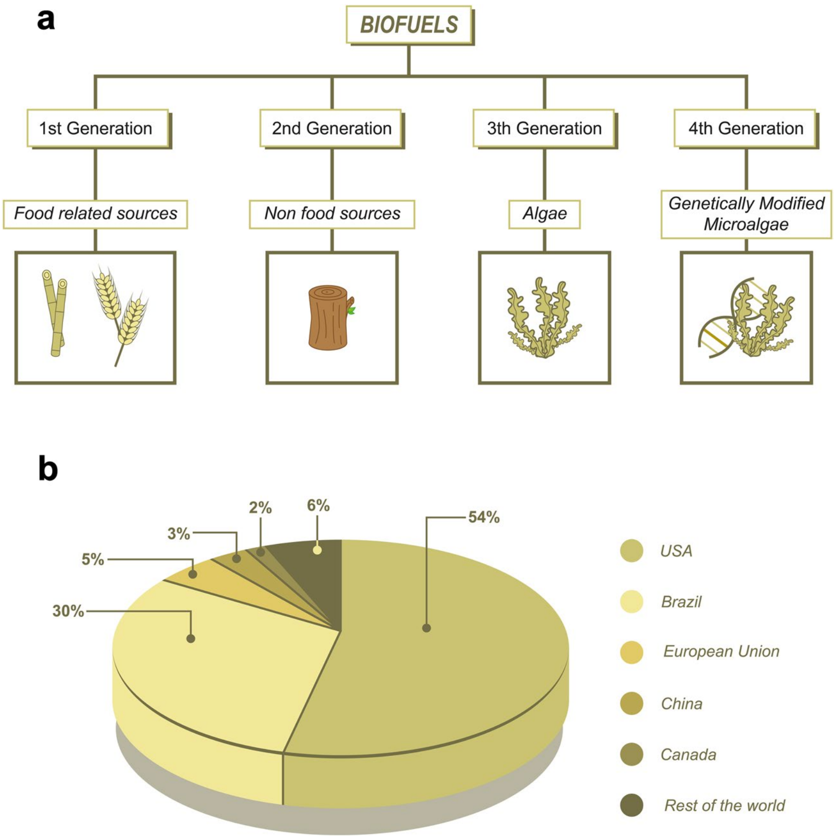


Fig. 2 **a** Biofuel categories and **b** main ethanol producers in the world. Data source: Renewable Fuels Association (RFA) (ethanolrfa.org/statistics/annual-ethanol-production), year: 2020

Biofuels can be gaseous (biogas), liquid (biodiesel, bioethanol, etc.), or solid [33]. The transport, storage, and high energy density of liquid biofuels make them more advantageous compared to solid and gaseous fuels [30]. Bioethanol and biodiesel are widely used as biofuel. Bioethanol contributes to 65% of the total biofuel production, impacting on the current economy [32]. This alternative fuel offers a range of environmental and socio-economic benefits due to its comparatively lower GHGs than fossil fuels (as CO_2 is recycled from the atmosphere during biomass production), potential to replace harmful fuel additives, and ability to create job opportunities for farmers and refinery workers. Bioethanol mixing with gasoline is possible and applicable to internal combustion engine vehicles [34]. Ethanol addition increases octane and reduces carbon monoxide (CO), volatile organic compounds (VOC), and particulate emissions of gasoline. Furthermore, via on board reforming to hydrogen, ethanol is also suitable for use in future fuel cell vehicles. As it was aforementioned, the biomass growth consumes as much CO_2 at it is formed during the combustion of bioethanol, making the net contribution to the greenhouse effect zero. Moreover, the use of bioethanol in older engines has the potential to decrease CO emissions from vehicles, thus improving air quality [35].

The USA and Brazil are at the forefront of global ethanol production, accounting for over 85% of the alcohol produced and commercialized worldwide. Figure 2b illustrates the percentage contribution of the worldwide leading ethanol producers, leading by the USA and Brazil followed by the European Union (EU), China, and Canada. In the USA, ethanol production primarily relies on corn, whereas in Brazil, it is predominantly derived from sugarcane. According to the European Renewable Ethanol Report in 2014, the most commonly used feedstocks for ethanol production in Europe were corn, wheat, and sugar beet [32].

Friendly Environmental Alternative Plastic Materials

Biopolymers are naturally occurring materials consist of repetitive monomeric units that covalently bonded to form larger molecules, such as cellulose, collagen, and alginates [36]. Biopolymers can be classified according to the origin (bio-based or synthetic) and its biodegradability. Bio-based polymers refer to those that are made entirely or partially from any type of renewable organic material of biological origin [37]. Biodegradable polymers are those which could microbiologically degraded into methane, CO_2, inorganic compounds, water, and biomass [38]. Biopolymers can divide into 3 groups: (i) biodegradable polymers produced from bio-based resources, (ii) biodegradable polymers derived from petrochemical resources, and (iii) non-biodegradable synthetized from bio-based monomers. Poly(lactic acid) (PLA), poly(hydroxyalkanoate)s (PHAs), and polysaccharides (cellulose, starch) are examples from the first group, poly(butylene adipate terephthalate) (PBAT) from the second one, and bio-poly(ethylene) (Bio-PE) from the third group [39].

Biopolymers can proceed from different sources like microbes, plants, and terrestrial and aquatic animals [40]. Some examples of plant sources are as follows: maize, wheat, sorghum, yams, cassava, potatoes, banana, tapioca, corn, cotton, etc. [25]. From animal sources can be mentioned cattle and corals, sponges, fish, lobster, and shrimp from marine animals. Algae, fungus, bacteria, and yeast are the most common microbiological sources.

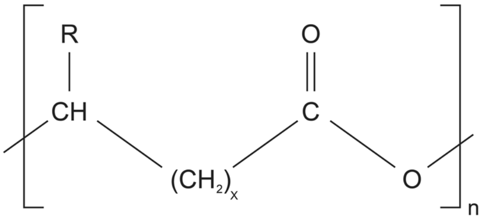
Among biopolymers, the most attractive for bioplastics industry are PHAs, which are a family of biopolyesters synthesized by numerous species of bacteria.

Structure and Classification of PHAs

PHAs are accumulated as intracellular granules under certain conditions when the external energy is over to the energy needs to maintain the process growth division or cell viability [41]. When the input of external energy is not enough, PHAs are depolymerized and metabolized to obtain carbon and energy source [42].

PHAs are linear polymers composed by several repetitions of the same monomer. More than 150 different types of monomers have been identified as constituents of PHAs [43]. The general structure of the repeating unit of PHAs and the more common substitutions of radical group are presented in Fig. 3. The *n* value will depend on the R group and the producer bacteria, varying between 10 and 30000 [44]. The most studies PHAs are as follows: poly(3-hydroxybutyrate) (PHB), poly(3-hydroxyvalerate) (PHV), and the copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). Among these biopolymers, PHB was the first class of PHAs characterized [45]. The copolymer PHBV is the result of the incorporation of 3-HV units in PHB chains, has lower melting point, and is more flexible and easier to thermal processing than PHB [46]. In accordance to the 3-HV content or the presence of other monomers, thermal and physical properties of the biopolymer will change and condition the copolymer applications. Feeding the microorganism with different substrates stimulates the production of different monomers, expecting to discover new PHAs.

PHAs can be categorized into different types according to the carbon chain length: short-chain length PHA (scl-PHA), medium-chain length PHA (mcl-PHA), and long-chain length PHA (lcl-PHA). The length of the monomer unit influences the polymer properties. In the case of scl-PHAs, which are composed of 3–5 carbon monomers, are thermoplastics with a high degree of crystallization, too rigid and



X=1	R=hydrogen	Poly(3-hydroxypropionate)
	R=metyl	Poly(3-hydroxybutirate)
	R=etyl	Poly(3-hydroxyvalerate)
	R=propyl	Poly(3-hydroxyhexanoate)
	R=pentyl	Poly(3-hydroxyoctanoate)
	R=nonyl	Poly(3-hydroxydodecanoate)
X=2	R=hydrogen	Poly(4-hydroxybutirate)
	R=metyl	Poly(4-hydroxyvalerate)
X=3	R=hydrogen	Poly(5-hydroxyvalerate)
	R=metyl	Poly(5-hydroxyhexanoate)
X=4	R=hexyl	Poly(6-hydroxydodecanoate)

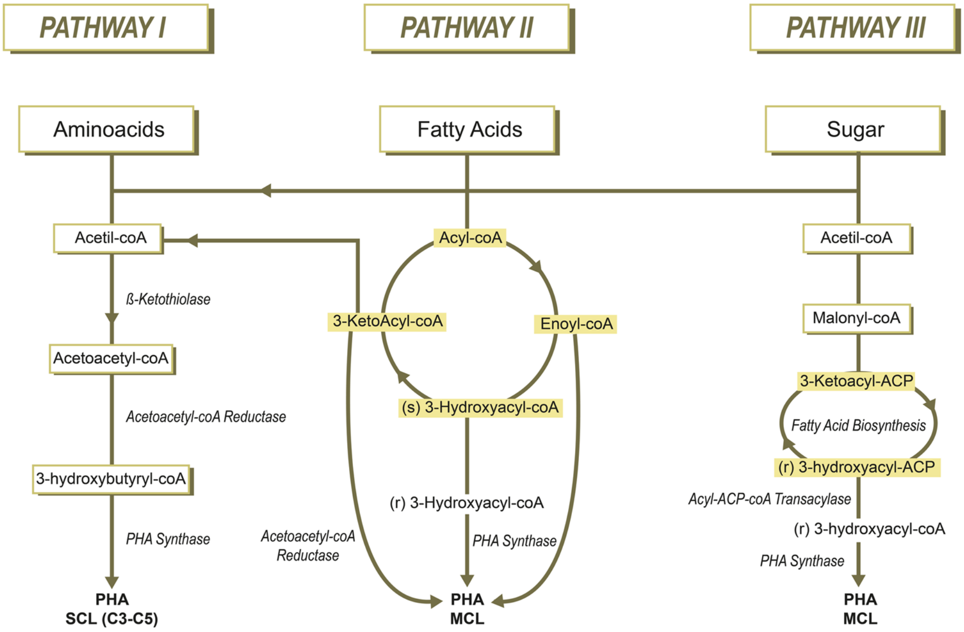
Fig. 3 General structure of PHAs and some identified comonomers. The number of consecutive CH$_2$ groups in the polymer backbone ranges from 1 to 4, $n=100$ to 30000

brittle, and are mostly used for the production of disposable items and food packaging [47]. On the other hand, mcl-PHAs, constituted by monomeric units of 6–14 carbons, are elastomeric, have a low degree of crystallization and melting temperature, and they have a low glass transition temperature and lower molecular mass when compared to scl-PHAs. Besides, mcl-PHAs are suitable for high value-added applications, such as surgical sutures, implants, and biodegradable matrices for drug delivery [48]. Finally, biopolyesters that contain monomer building blocks of 15 or more than 15 carbons are lcl-PHAs [49]**.** A PHA will be short- or medium-chain polymer accordingly to the enzyme responsible for the synthesis (synthase) since this is specific to the substrate and can act on monomers with different number of carbon atoms. Thereby, PHA chemical composition will depend on the substrate, the PHA-synthase enzyme, and the metabolic pathway involved [50].

PHA Biosynthetic Pathways

The monomer composition is related to the used carbon source. There are three well-known PHA biosynthetic pathways (Fig. 4). In acetoacetyl coenzyme A (acetoacetyl-coA) pathway (pathway I), 2-acetyl-coA is produced from either fatty acids, sugar, or amino acid and converted to acetoacetyl-coA by the enzyme-β-ketothiolase [51]. Acetoacetyl-coA reductase acts on acetoacetyl-coA to form 3-hydroxybutyryl-coA, which is later polymerized by PHA synthase to produce PHB. This pathway describes the production of scl-PHAs that are composed of 3–5 carbon monomers. In the beta-oxidation cycle pathway (pathway II), fatty acids are converted to

Fig. 4 PHA biosynthetic pathways



enoyl-coA. This is catalyzed to R-3-hydroxyacyl-coA by R-3-hydroxyacyl-coA hydratase and later converted to PHA by PHA synthase. This pathway describes mcl-PHA synthesis. The third pathway, in situ fatty acid synthesis (pathway III), is produced from simple carbon sources such as glucose, fructose, gluconate, glycerol, ethanol, and acetate. These carbon sources are generally present in inexpensive organic wastes such as glycerol, a by-product of biodiesel production [52]. These carbon sources are convert into acetyl-coA, and later into malonyl-coA culminating in 3-hydroxyacyl-acyl carrier protein (ACP) [53]. 3-hydroxyacyl-ACP is transform to 3-hydroxyacyl-coA for the synthesis of PHA by the enzyme 3-hydroxyacyl-ACP-coA tranferase [51]. This pathway also allows to obtain mcl-PHA.

Bacterial Species That Accumulate PHAs

PHAs are produced by a variety of Gram-positive and Gram-negative bacteria. Comparing both bacteria, Gram-negative have a greater capacity to accumulate PHAs [54]. However, the principal disadvantage of the PHAs obtaining from this kind of bacteria is the presence of lipopolysaccharide (LPS) endotoxins in the bacteria's outer cell membrane which can elicit a strong inflammatory response when is in direct contact with humans. For this reason, these PHAs are unsuitable for biomedical applications [55]. Removal of LPS endotoxins can be done; however, this practice increases the overall cost of PHA production and generates changes in the biopolymer properties (i.e., reduction in molecular mass and polydispersity). Instead, Gram-positive bacteria have lack of LPS, making them a better source of raw material to obtain PHAs for biomedical applications [56].

Bacteria that are used for PHA production can be classified into two groups. The first requires limitation of essential nutrients such as nitrogen, phosphorus, and magnesium, as well as the presence of excess carbon source for the efficient biopolymer synthesis [57]. Bacteria included in this group are *Cupriavidus necator*, *Bacillus* sp., *Protomonas extorquens*, and *Protomonas oleovorans*. On the other hand, the second group of bacteria does not require nutrients limitation and can accumulate PHA during exponential growth phase. *Alcaligenes latus*, a mutant strain of *Azotobacter vinelandii*, and a recombinant strain of *E. coli* are examples of this group. It is important to highlight that *Pseudomonas* is the only reported species to produce long carbon chain PHAs [58]. Several species accumulate PHAs, including *Cupriavidus necátor* [59–68], *Bacillus* sp. [69–78], *Alcaligenes latus* [79–81], *Azotobacter* sp. [82–87], *Aeromonas* sp.[88], *Burkholderia* sp. [89–91], *Psedomonas* sp. [92–99], *Halomonas* sp. [100–104], *Haloferax* sp. [105–111], and *Recombinant E. coli* [112–118].

PHA Structure–Property Relationship

PHAs are semi-crystalline polymers that exhibit a wide variety of mechanical properties depending on their composition and type of constituent monomers [119]. Scl-biopolymers are stiff, brittle, and possess a high degree of crystallinity in the range of 60–80% [120]. Besides, mcl-PHAs are crystalline elastomers, they are flexible and elastic materials, have low crystallinity (20–25%), low tensile strength, and high elongation at break [121]. PHB is the most studied member among the PHAs. Its mechanical properties, such as Young's modulus and tensile strength, are very similar to poly(propylene), although the elongation at break is lower than other synthetic polymers [122]. The main disadvantage of PHB is the high degree of crystallinity leading to brittleness. The incorporation of other HAs such as hydroxyvalerate (HV), hydroxyhexanoate (HHx), 3-hydroxypropionate, and 4-hydroxybutyrate, to form copolymers can reduced its brittleness, become less crystalline and more flexible [123].

PHA thermal properties are expressed in terms of glass transition temperature (Tg) for the amorphous phase and the melting temperature (Tm) for the crystalline domain. The increase of the side chain length from 1 to 7 carbons decreases Tg; meanwhile, a change from 4 to 7 carbon side chain length produces a Tm increase from 45 to 69 °C [121]. Melting temperature can be improved by the incorporation of other HAs, for example, the increasing 3HV content on the copolymer PHBV decreases the melting temperature without any considerable variation in the thermal degradation [124]. In summary, the thermal properties can be controlled by adjusting the 3HV content incorporated in the copolymer during fermentation. This strategy gives to the copolymers a larger thermal processing window without causing thermal degradation. In Table 1 are reported the main mechanical and thermal properties of diverse PHAs in order to show the wide variability.

The thermal and physical properties of these PHAs can be controlled during fermentation by feeding different substrates and in different ratios. The properties of PHAs are also influenced by the fermentation time. During the late stationary phase of cultivation, the presence of endogenous PHA depolymerases can lead to the degradation of intracellular PHAs, resulting in a significant decrease in the polymer molecular weight. Additionally, factors unrelated to biosynthesis, such as the extraction technique, the type of extraction agent, and the purification method, can also modify the properties of the biopolymer.

Another property that depends on the PHA structure is the biodegradability. The biodegradability of PHAs in natural environments such as soils, sea, and lake waters is a process that is generally influenced by several factors such as microbial population, temperature, humidity level, pH, nutrient concentration as well as biopolymers composition,

Table 1 Main thermal and mechanical properties of PHB and other PHA copolymers [120]

Polymer	Melting temperature	Young's modules (GPa)	Tensile strength (MPa)	Elongation at break (%)	Glass transition temperature (°C)
P(3HB)	173–180	3.5–4	40	3–8	5–9
P(3HB-co-3HV) (3 mol% 3HV)	170	2.9	38	nr	nr
P(3HB-co-3HV) (9 mol% 3HV)	162	1.9	37	nr	nr
P(3HB-co-3HV) (14 mol% 3HV	150	1.5	35	nr	nr
P(3HB-co-3HV) (20 mol% HV)	145	1.2	32	nr	−1
P(3HB-co-3HV) (25 mol% 3HV)	137	0.7	30	nr	nr
P(4HB)	53	149	104	1000	−50
P(4HB) (3 mol% 4HB)	166	nr	28	45	nr
P(4HB) (10 mol% 4HB)	159	nr	24	242	nr
P(4HB) (16 mol% 4HB)	130	nr	26	444	−7
P(4HB) (64 mol% 4HB)	50	30	17	591	−35
P(4HB) (90 mol% 4HB)	50	100	65	1080	−42
P(3HB-co-3HA) (6 mol% 3HA)	133	0.2	17	680	−8
P(3HB-co-HP) (67 mol% HP)	44	nr	nr	nr	−19
P(3HB-co-3HHx)	52	nr	20	850	−4

nr, not reported

crystallinity, and structure [125]. The biodegradation process of PHAs can be differentiated into two categories: intracellular and extracellular degradation. Intracellular biodegradation occurs when PHAs stored in the cell cytoplasm are hydrolyzed for be used as an energy reserve when no other carbon source is available in the medium. Intracellular biodegradation is a long process compared to biosynthesis. Meanwhile, extracellular biodegradation is the most relevant and important. Many microorganisms, including bacteria and fungi, have the ability to secrete enzymes capable of hydrolyzing PHAs, which are called PHA hydrolases and PHA depolymerases. PHA degradation is an interesting process, not only to reduce the excessive accumulation of plastic waste in the environment, but also for the possibility of using by-products of PHA hydrolysis for the synthesis of other polymers with a wide range of properties [126].

Industrial PHAs

PHAs have been commercially produced since the 1980s; however, their application stagnated due to low petroleum prices. In early 2000s, the increase in petroleum prices sparked a renewed industrial interest in PHAs. Subsequently, new plants were established in China, the USA, Italy, and Brazil. Industrial-scale PHA production takes place in fermentation reactors and the processes involve various factors

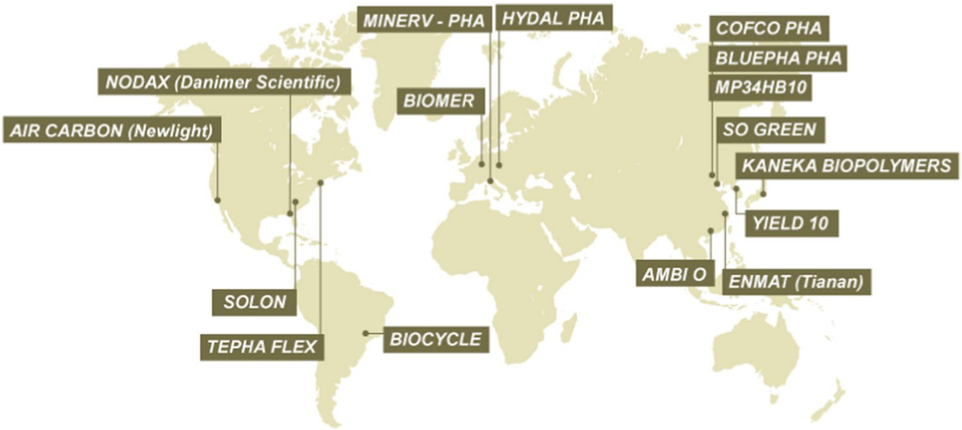


Fig. 5 Worldwide leading global PHA-producing plants

such as substrate selection, bacterial strains, integration into existing bioprocessing facilities, and the specific type of produced PHA. Figure 5 illustrates the current leading global PHA-producing plants.

Although biopolymers account for only 1% of the global polymer production derived from petroleum, the industry is experiencing a quick growth. The market size value of PHAs in 2020 was estimated to be US$ 168.9 million, and it is projected that the market will reach a value of US$ 440 million by 2031. This indicates a compound annual growth rate (CAGR) of 9.2%. Furthermore, the recent production of PHAs is already contributing to an existing industrial value chain that is likely to expand with greater availability of commercial PHAs [127].

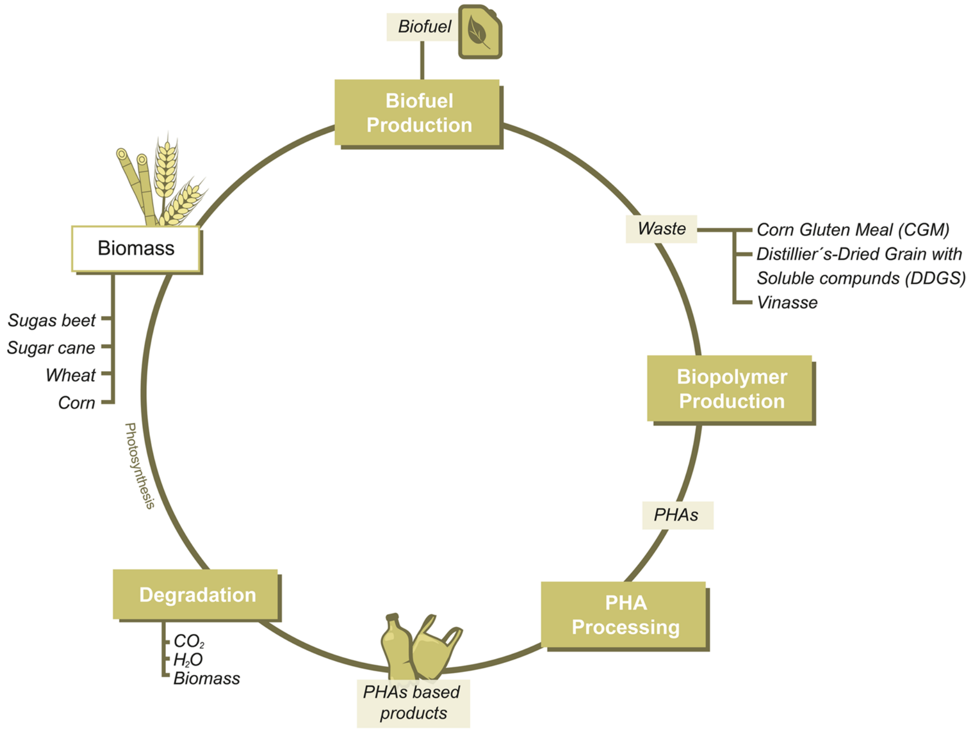
Biotechnological Industry: Integrated Systems for Biofuel and Biopolymer Production — Recent Advances in PHAs

The raw materials and chemicals using as sources of organic matter for commercial PHA production are expensive which entails high costs at industrial scale [128]. To become an economically attractive alternative to fossil-fuel plastic production, these costs must be reduced. Snell and Peoples [129] made an analogy between the petroleum and the biotechnological industries since both of them can cover society's needs for fuels and plastics. Nevertheless, biotechnological industry, which integrates biorefinery and plastic production, will reduce the environmental negative impact of the fossil fuels and synthetic plastics. This integration involves the use of the biorefinery waste streams as raw materials to obtain biopolymers via microbial fermentation (Fig. 6). Depending on the used biomass as raw material to obtain biofuel, different residues are generated. Corn gluten meal (CGM) and distillers' dried grains with soluble compounds (DDGS) are the major high nutrient by-products derived from corn ethanol production [130]. The production of ethanol from sugarcane or sugarbeet generates vinasse, a residue rich in organic matter and minerals [131].

These waste streams are rich in nutrients that several microorganisms can employ to produce biopolymers through fermentation processes. Microbial biopolymer polymers include intracellular and extracellular polymers. Extracellular polymeric substances (EPS) are materials secreted by bacterial consortia during cell metabolism and form a complex and diverse biopolymeric matrix consisting of proteins, exopolysaccharides, lipids, glycoprotein, etc. [132]. Intracellular polymers are storage molecules such as polysaccharides, polyamides, polyesters, and polyphosphates [133]. Among microbial biopolymers, the most attractive for bioplastics industry are the PHAs, which are a family of biopolyesters synthesized by numerous species of bacteria.

Various strategies have been proposed at the laboratory scale to enhance the profitability and market viability of PHA production. Among these strategies, utilizing industrial by-products and waste streams as carbon sources for PHA production has emerged as a highly promising option. This

Fig. 6 Integration of the biorefinery waste streams as raw materials to obtain biopolymers via microbial fermentation



approach involves utilizing materials such as agricultural feedstocks, waste plant oils, and wastewaters as sustainable sources of carbon for PHA synthesis. By employing waste streams as raw materials, a more environmentally friendly approach can be achieved. However, further technological advancements are necessary to facilitate the large-scale implementation of this method and enable its successful application at pilot and industrial settings. In Table 2 are reported several academic works that employed diverse residue wastes derived from biorefineries as carbon sources to produce PHAs by different bacterial strains. Besides, it were mentioned the biopolymer content and accumulation percentage.

Ecological Impact of PHA Production in Integrated Systems

A biorefinery produce biofuels, energy, and chemicals from biomass conversion processes [148]. The design of a sustainable biorefinery should take into account various factors including competition with biomass and other raw material resources, water usage, product quality, land usage, GHG emissions, and impacts on biodiversity. Biorefineries aim to employ zero-waste production processes and prioritize high energy efficiency, resulting in the manufacturing of products with minimal carbon and water footprints [149]. The impacts of bio-based materials should be quantified by applying life cycle assessment (LCA) to evaluate the potential environmental impact not only of the final products but also the production process. LCA can provide solid, comprehensive, and quantifiable information about the ecological performance of the products and processes, highlighting their environmental advantages. Besides, LCA can allowing detecting critical points that should be optimize to achieve more green processes. LCA studies for PHA production are not conclusive about the ecological performance of these biopolymers, comparing with their fossil competitors. Some studies reveal that PHAs can be reduce the environmental impact respect to synthetic and non-biodegradable polymers, especially if industrial and ecological by-products and wastes as well as clean energy are used for PHA production [150]. LCA studies are not unequivocal in their results since it can be employed different normative basis of evaluation methods and different contexts of the technologies compared. Besides, LCA depends on the inherent characteristics of each production process, mainly the carbon source; the fermentation, extraction, and purification steps; and the energy source. However, LCA is a potent tool for supporting technological development and design, as well as to identify ecological hot spots and assess optimization potentials.

The fact that PHA production carries out under the biorefinery concept reduces the environmental impact of biofuels and biopolymers obtained from these integrated systems. In order to reach this reduction, it is essential that biopolymer production plants are located near the biofuel refineries that allows minimize the impact associated to the by-products transport. In the case of biofuels, the exploitation of residual wastes helps to solve the negative impact of their final

Table 2 Diverse residue wastes derived from biorefineries to produce PHAs by bacterial strains

Substrate used	Strain	PHA (g/L)	PHA (%)	References
Sugarcane bagasse	*Bacillus cereus*	0.3	23.1	[134]
Sugarcane bagasse hydrolysate	*Burkholderia glumae*	nr	14.9	[90]
Sugarcane bagasse	*Bacillus* sp.	5	55.5	[135]
Sugarcane bagasse + corn steep liquor	*Lysinibacillus* sp.	5.31	61.5	[136]
Sugarcane bagasse	*Klebsiella pneumonia* 61	9	nr	[137]
Sugarcane bagasse	*Bacillus safensis EBT1*	nr	nr	[138]
Sugarcane bagasse	ART_MKT2E	0.088	55	[139]
Pretreated vinasse	*Haloferax mediterranei*	19.7	70	[140]
Vinasse	*Cupriavidus necator*	1.33	26	[141]
Vinasse	*Chlorella* sp.	nr	nr	[142]
10% raw vinasse	*H. marismortui*	2.8	23	[143]
100% pre-treated vinasse		4.5	30	
10% raw vinasse	*Bacillus megaterium*	0.25	25.5	[144]
Sugarcane vinasse and molasses	*Cupriavidus necator*	11.7	56	[145]
Sugarcane molasses and vinasse M/V: 25/75	*Cupriavidus necator*	3.17	85.9	[146]
Wheat waste	*Ralstonia eutropha*	7.85	74	[147]

nr, not reported

% PHA, accumulation is the amount of biopolymer in relation to the dry cell weight

disposal. Respect to PHA production, the use of inexpensive residual wastes as carbon source and clean energy also reduce the environmental impact.

Future Perspectives

PHAs present a series of remarkable advantages. Firstly, their renewable origin makes them an environmentally friendly option as they are derived from sustainable sources. Additionally, being biodegradable, they naturally decompose without leaving harmful residues. This makes them a favorable alternative to non-biodegradable synthetic polymers that can have a negative impact on the environment. Another notable advantage is their ability to offer similar properties to synthetic polymers, making them a versatile alternative with applications in various fields. For example, in the biomedical industry, PHAs can be used in the manufacturing of biocompatible medical devices and implants that safely degrade in the human body. They also excel in the creation of self-care products, such as biodegradable packaging and environmentally friendly cosmetics.

Overcoming the challenges to scale up PHA production from waste requires addressing several aspects. Firstly, the variability in waste composition is a significant challenge as it can affect the quality and properties of the obtained biopolymers. Comprehensive research is needed to understand and optimize the conversion process of different types of waste into PHAs. Additionally, ensuring a constant availability of the required waste for the operation of the PHA production plant is crucial. This involves establishing long-term agreements with agro-industrial waste suppliers and developing an efficient supply chain that guarantees a continuous availability of raw materials.

The integration of the PHA production process into the bioethanol production plant is another challenge to consider. It requires optimizing the existing infrastructure and implementing strategies to efficiently couple both processes, thereby maximizing resource utilization and reducing operating costs. The pretreatment of waste is a critical stage to maximize the efficiency of substrate conversion into PHAs. It is necessary to develop and optimize suitable pretreatment techniques for each type of waste to remove impurities and facilitate the efficient transformation of substrates into biopolymers. Moreover, optimizing the operational variables of the PHA production process, such as temperature, nutrient concentration, pH, and fermentation times, is essential. This will ensure high yields and consistent quality of the final product.

In conclusion, producing PHAs from agro-industrial waste and bioethanol by-products offers a promising sustainable alternative to non-biodegradable synthetic polymers. Although technical and logistical challenges persist, research and development in this field are continually advancing. As these challenges are overcome and efficient solutions are implemented, sustainable PHA production can be achieved, promoting a circular economy and reducing dependence on non-renewable fossil resources.

Conclusion

The integration of biopolymer production and biofuels is an interesting alternative to current petroleum refinery. Among biopolymers, PHAs are promising because they have some properties suitable for accessing the markets currently served by petroleum-based plastics. In this review, it was demonstrated the feasibility to synthetize PHAs from different waste streams of the bioethanol production, following the biorefinery concept. The valorization of low-cost waste streams to obtain high-value added biopolymers is not only an economically attractive proposal but also a green initiative to a more sustainable process. Nevertheless, it is important to carry out environmental studies to assure that used methods and techniques really minimize the ecological impact and the final products are truly green and sustainable. As society's concern for the environment and sustainability continues to grow, the synergy between biopolymer production and biofuels offers a promising solution, merging economic benefits with ecological consciousness.

By utilizing waste materials from bioethanol production as raw materials, the reliance on non-renewable resources is reduced while simultaneously fostering a circular economy, where waste is transformed into valuable resources. Moreover, the production of biopolymers from renewable sources, such as bioethanol waste, contributes to the reduction of greenhouse gas emissions and the overall carbon footprint associated with conventional plastic manufacturing. This transition towards more sustainable materials is crucial in addressing climate change and ensuring a cleaner and healthier future for generations to come.

Abbreviations CO_2: Carbon dioxide; GHG: Greenhouse gas; CO: Carbon monoxide; VOC: Volatile organic compounds; EU: European Union; PLA: Poly(lactic acid); PHA: Poly(hydroxyalkanoate); PBAT: Poly(butylene adipate terephthalate); Bio-PE: Bio-poly(ethylene); PHB: Poly(3-hydroxybutyrate); PHV: Poly(3-hydroxyvalerate); PHVB: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate); scl-PHA: Short-chain length poly(hydroxyalkanoate); mcl-PHA: Medium-chain length poly(hydroxyalkanoate); lcl-PHA: Long-chain length poly(hydroxyalkanoate); LPS: Lipopolysaccharide; Tg: Glass transition temperature; Tm: Melting temperature; CGM: Corn gluten meal; DDGS: Dried grains with soluble compounds; EPS: Extracellular polymeric substances; LCA: Life cycle assessment

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Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethical Approval This declaration is "not applicable."

Competing Interests The authors declare no competing interests.

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